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Characteristics and Identification Methods of Veterinary Important Mange Mites

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Abstract

Mites are of among the serious skin parasites of both animals and humans worldwide. The disease they cause is called mange or scabies. Mange mites of medical importance are categorized into two major groups. One group is the burrowing type that found deeply under the skin, and affects skin follicles and sebaceous glands. This group consists of Demodex, Sarcoptes, etc. The other group is no burrowing type and found on the surface of the skin. Psoroptes and Chorioptes are the well-known non burrowing mites. Each mite has its own unique morphological and behavioural characteristics. Hence for effective therapy and prevention and control of the disease, identification and characterization of mites is mandatory. Therefore, this mini-review highlights mites' characterization and identification using morphological, serological and molecular methods.

Introduction

Mange (scabies) is a parasitic skin disease caused by microscopic mites. Mites are obligate and permanent parasite belonging to the order Acarines. Two different mange mites cause this skin disease in animals. One lives just under the surface of the skin (e.g. psoroptic mange), while the other resides in the hair follicles (e.g. demodectic mange). In other words, mites causing mange of animals of veterinary importance usually belong to two broad families: borrowing and non-borrowing. The borrowing group consists of mainly Sarcoptidae and Demodicidae while the non-borrowing groups are Psoroptidae and Chorioptidae. Although both mites share similar characteristics, there are also important differences. It is important not to confuse the two types of mange because they have different causes, treatments, and prognoses [1].

Similar in appearance to ticks but much smaller, mites have bulbous, round, or pill-shaped bodies. Classified as arachnids, mites have eight jointed legs. Their size varies by species, but most mites are usually invisible to the naked eye. The largest mites measure about 6 mm long, while the smallest are about 0.1 mm. The colour of mites varies greatly as well; most mites appear tan, brown, or reddish-brown, but some species are bright red, blue, or green in colour [2].

Characteristics of Mange Mites

a. Sarcoptic Mange (Scabies)

*Sarcoptesscabiei*var*bovis* is a highly contagious disease spread by direct contact between infested and naive animals or by contaminated fomites. Lesions caused by this burrowing mite start on the head, neck, and shoulders and can spread to other parts of the body. The whole body may be involved in 6 weeks. Pruritus is intense, and papules develop into crusts; the skin thickens and forms large folds. *S. scabiei*var*bovis* can also be transmitted to people and result in a transient, self-limiting dermatitis [3].

b. Psoroptic Mange

Psoroptic mange in animals is caused by infestation with *Psoroptes ovis*. Current taxonomic and systematic classification of *Psoroptes spp* indicates that *P. ovis* and *P. cuniculi* (ear canker in rabbits, ear mange in sheep and goats) are strains or variants of the same species, with *P. ovis* being found primarily on the backs and flanks of infested animals and *P. cuniculi* in the ears. *P. ovis* is not zoonotic. *P. ovis* is a non-burrowing mite that lives on the skin surface. All stages of the mite are found on the host, and transmission is through direct contact of infested and susceptible hosts. Transmission is also possible through contact with contaminated environments or fomites, because *P. ovis* can survive off the host for ≥2 weeks under the right conditions [3].

c. Chorioptic Mange

Chorioptic mange in cattle is caused by infestation with *Chorioptesbovis* or *C. texanus*. Species of *Chorioptes* are not host specific, and *C. bovis* can be found on domestic ruminants and horses throughout the world. Chorioptic mange caused by infestation with *C. bovis* is the most common type of mange in cattle in the USA. *C. texanus* has been reported on cattle from Brazil, China, Germany, Israel, Japan, Malaysia, South Korea, and USA. *C. bovis* and *C. texanus* are not zoonotic. *C. bovis* live on the skin surface and do not burrow. Life cycle stages include egg, larval, two nymphal, two female, and one male stage. Eggs are deposited on the skin, and secretions from female mites help secure eggs to the surface of their hosts. Eggs require 5-6 days to hatch, whereas each larval and nymphal stage requires 3-5 days for development. The entire life cycle may be completed in 21-26 days and depends on temperature and humidity. Transmission is by direct contact of infested and naive hosts. *C. bovis* can live off their host for up to 3 weeks and can be transmitted to cattle through contact with contaminated fomites and housing. Chorioptic mange is less pathogenic than sarcoptic or psoroptic mange in cattle [3].

d. Demodectic Mange (Follicular Mange)

Three species of *Demodex* are known to infest cattle: *D. bovis*, *D. ghanensis*, and *D. tauri*. *D. bovis* is the most common and infests hair follicles of cattle worldwide. *D. ghanensis* infests meibomian glands of cattle from Ghana, and *D. tauri* has been recovered from hair follicles and sebaceous glands of cattle from Czechoslovakia. Species of *Demodex* are very host specific and typically occur either in hair follicles or dermal glands, and they are not zoonotic. *Demodexspp*are

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unique among parasitic mites, because they are elongated with short, stumpy legs. Their distinct morphology is a presumed adaptation to living in hair follicles and sebaceous glands of their hosts. All life cycle stages are found on the host and include egg, larvae, two nymphs, and adults. These mites feed on sebum, protoplasm, and epidermal debris. Transmission of *D. bovis* occurs through close contact of infested and naive hosts, with the transfer of mites from infested dams to neonates being the primary route [3].

Identification Methods

a. **Morphological Identification Methods**

i. Sarcoptic mange

Sarcoptes is round in outline and up to 0.4mm in diameter withprominent dorsal pegs and spines. And its most important recognition characters are the numerous transverse ridges and triangular scales on the dorsum, features possessed by no other mange mite of domestic mammals. They have short legs and dorsally the legs only just project beyond the edge of the body and the posterior two pairs of legs do not extend beyond the body margin at all.Pulvillus is originated on a stalk-like or unsegmentedpretarsus on 1st and 2nd pairs of legs. The male is about 250 μm in length and is smaller than mature female which is about 400 to 430 μm in length. The dorsal surface of the body of *Sarcoptesscabiei* is covered with transverse ridges but also bears a central patch of triangular scales. The dorsal setae are strong and spine like. The anus is terminal and only slightly dorsal (Figure 1) [4].

ii. Demodectic mange

Demodex has an elongated tapering body (cigar shape), up to 0.2mm long, with four pairs of stumpy legs anteriorly. Setae are absent from the legs and body. The legs are located at the front of the body so that the striated-opisthosoma forms at least half the body length. The mouth parts consist of paired palps and chelicerae, and an unpaired hypostome [4].

iii. Psoroptic mange

Psoroptes is a typically non burrowing mite, up to 0.75mm, oval in shape, and with all the legs projecting beyond the body margin. Its most important recognition features are the pointed (conical) mouth parts, the rounded abdominal tubercles of the male and the three jointed pedicles (pretarsi) bearing funnel-shaped suckers on most of the legs (on 1st, 2nd and 4th pairs of legs) (Figure 1) [4].

iv. Chorioptic mange

The mouth parts are distinctly rounder, the abdominal tubercles of the male are noticeably truncate and the pedicles are short and unjointed (unsegmented), with cup-shaped sucker. Adult female *Chorioptes bovis* are about 300 μm in length, considerably smaller than Psoroptesovis **(Figure1)** [4].

b. **Serological Identification Methods**

Several serological assays for scabies have been developed over the past decades. In 2007, Casais et al. [5] developed an enzyme-linked immunosorbent assay (ELISA) based on identification of a 642 amino acid polypeptide using a recombinant *S. scabiei* var. *hominis* library. After insertion of the encoding complementary DNA in *Escherichia coli*, antiserum was raised and used in Western blots of serum from chamois with scabies. The technique proved to be highly sensitive (100%) and specific (97%) in identifying infected animals. In 2010, Walton *et al* [6] developed a quantitative immunoglobulin E (IgE) inhibition assay for human use that identified Ig Eimmunoreactivity of scabies mite antigens based on recombinant-produced *S. scabiei* cysteine or serine proteases and apolipoproteins. These antigens are located in the mite cuticle or gastrointestinal tract. They found significant IgE levels in patients with scabies compared with normal control subjects and, by using inhibition ELISA, no or minimal cross-reactivity with house dust mite antigens was observed. With the apolipoproteinSsag 1.2, the sensitivity of the IgE assay was 88% and specificity was 100%. They tested patients with different clinical forms of scabies and found that greater IgE reactivity was seen for mite apolipoproteins with serum from patients with the crusted forms compared with normal scabies.

In 2015, Arlian et al. [7] prepared aqueous extracts from *S. scabiei var. canis* as well as from common house dust mites. The antigen was then tested using sera from 91 patients and screened for IgA, IgE, IgG, IgM and IgD antibodies to *S. scabiei*. However, antibodies were found to cross react with house dust mites, including *Dermatophagoidesfarinae, Dermatophagoidespteronyssinus* and *Euroglyphusmaynei*. Later, a further assay was developed with a recombinant *S. scabiei* actin-associated cofilin protein from rabbit scabies which is present in the splanchnic area, but not the exoskeleton, and shares 90% identity with *D. farinae*cofilin. One of the serological assays developed using this antigen of *S.scabiei* was an indirect ELISA that was tested using rabbit serum from rabbits infected with scabies and uninfected controls as well as some with *Psoroptescuniculi* and *Cysticercosispisiformis* infestations. This method showed 83.33% sensitivity and 87.9% specificity [8].

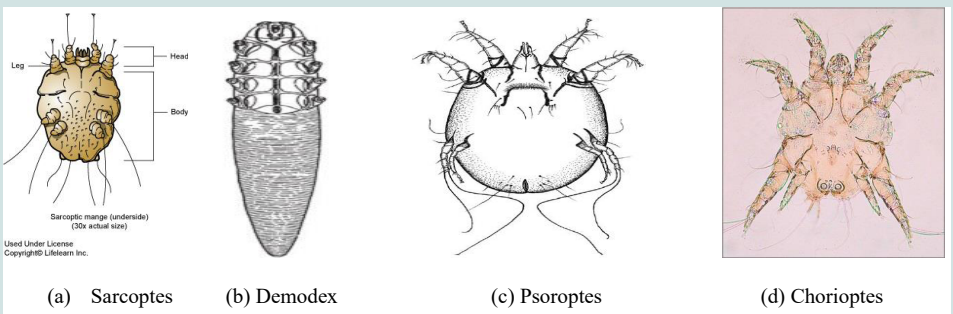

Figure 1: Morphological features of the four important Mange mites: (a) Sarcoptes (b) Demodex (c) Psoroptes and (d) Chorioptes. Source: Abriham [4].

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A dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) was also developed based on a recombinant Sar s 14.3 major scabies antigen. No cross-reactivity was noticed using the house dust mite homologue Der p 14, and in a study of infested and control human subjects it proved to be a highly sensitive (100%) and specific (93.75%) method for scabies diagnosis in clinical settings [8].

Another cloned protein that has been investigated is a *S. scabiei* tyrosine kinase (SsPTK), which is mainly located in the mouth part region of the scabies mite. It was evaluated in a rabbit model and was found to have a sensitivity of 95.2% and a specificity of 94.1%; it was also able to detect infection early in its course after 1 week.Other potential protein targets that have been assessed in experimental scabies models are triosephosphateisomerase, calmodulin and chitinase. In summary, detection of targeted scabies antibodies using immunoassays has shown promise, but the antigens targeted by these tests have not been adopted for use in immunologically based antigen detection assays [8].

c. **Molecular Identification Methods**

Scabies can also be identified by molecular methods that select different targets, including microsatellites, ITS-2 ribosomal DNA (rDNA), mitochondrial 12S/16S rRNA and *S. scabiei myosin* heavy chain genes. Additionally, an attempt was made recently to characterize the parasite proteins in order to establish the diagnosis using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). This work has now generated details of many new molecular targets for subsequent proteomic work [8].

v. DNA Isolation

S. scabiei DNA can be extracted using several different protocols. This can be accomplished directly from human samples such as skin biopsiesand swab specimens. DNA can be extracted with commercial kits such as the Qiagen Tissue Kit (Qiagen, Hilden, Germany), QIAamp DNA minikit (Qiagen), Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany)and silica magnetic NucliSENSeasyMAG kit (bioMérieux, Marcy-l'Étoile, France) [8].

vi. Conventional Polymerase Chain Reaction (PCR)

In 2001, Bezold et al. were able to identify *S. scabiei* in skin samples of a patient using primer sequences from highly conserved regions of *S. scabiei* microsatellite 15 (Sarms15). Later, in 2015, a further *S. scabiei*–specific conventional PCR was developed. This PCR was based on the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene of *S. scabiei*, in which coding regions were selected that had no homology to any known sequences of potentially cross-reactive mites, including *Demodexfolliculorum*, *Demodexbrevis*, *Dermatophagoidespteronyssinus*, *Dermatophagoides farina*, *Dermanyssusgallinae*, *Tyrophaguputrescentiae* and *Cheyletusmalaccensis*. PCR primers scabF1 and scabR2 appeared to be specific for *S. scabiei* and generated a 250 bp product. The sensitivity and specificity were 100%. The assay detected all 17 microscopy-positive patients and confirmed the diagnosis in an additional 12 patients. All these additional patients had compatible skin lesions and responded to antiscabetics [8].

In further work, another conventional PCR was developed by Angelone-Alasaad*et al* [9] in 2015 for the rapid diagnosis of scabies. Based on mitochondrial 16S rDNA, a primer set was developed that generated a PCR product of 135 bp with *S. scabiei.* This was used for the diagnosis of animal scabies, using skin samples taken from six different animal species with confirmed sarcoptic mange (scabies) [8].

Recently Delaunay *et al*developed a PCR assay for identification based on the ITS-2 region that showed somewhat lower sensitivity to previously developed PCR assays in human scabies using a different sampling technique of swabbing the skin over the wrist and interdigital spaces of infested patients. Of 87 patients with dermatoscopically confirmed scabies, 33 had positive scabies PCRs, with a sensitivity of 37.9% and a negative predictive value of 61.7%. The authors pointed out the potential use of this as a screening method in scabies outbreaks [8].

vii. Real-time Polymerase Chain Reaction (RT-PCR)

To discontinue the use of gel electrophoresis and to generate results more quickly for some causative agents, real-time PCR is appropriate. In 2015, a novel quantitative PCR (qPCR) targeting a 121-bp fragment of the *cox1* gene of *S. scabiei* was designed and evaluated using samples collected from human skin at different body sites before and after medical treatment. The newly developed qPCR could also be used to monitor treatment response, as the number of *S. scabiei* DNA copies was higher before the treatment and decreased after initiating treatment, becoming undetectable at days 14, 21 and 28 after the start of treatment [8].

As a further approach to rapid detection, a TaqMan real-time PCR assay was developed in 2015 by Angelone-Alasaad*et al* [9]. They used the assay as a diagnostic method for sarcoptic mange in different animal species. In this assay, a specific probe for *S. scabiei* was developed using amplification of 135 bpfrom mitochondrial 16S rDNA. The technique was highly sensitive and no cross-reactivity was observed. It was also more sensitive than endpoint PCR, as a minimum amount of *Sarcoptes* genomic DNA of 10 pg/µLwas needed compared with 80 pg/µL for the conventional assay [8].

In 2020, Bae et al. [10] developed an in-house reverse transcription PCR assay based on the *cox1* gene of *S. scabiei*, a 196 fragment using primers cox1F and cox1R with a cox1P2 probe. The authors used the IACS criteria as their case validation method. A total of 47 patients were tested; 33 had a suspected diagnosis of scabies, 10 had unrelated disease and 4 were healthy individuals. Of the 33 suspected cases, 22 had microscopy-proven scabies, 2 had clinically diagnosed scabies, 6 had suspected scabies and 3 were negative. Samples were obtained by scraping lesional skin. The assay showed a sensitivity of 86% in confirmed scabies cases, 83% in confirmed but clinically diagnosed scabies and 80% in clinically suspected scabies and 100% specificity. The results also matched the declining certainty of the diagnosis based on the IACS clinical criteria [8].

viii. Isothermal Amplification Techniques

The downside of using PCR-based identification tools is that there is still the need to use thermocyclers to amplify the DNA. In the past few years, isothermal amplification techniques have been developed to overcome this shortcoming. These include loop-mediated

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isothermal amplification (LAMP), rolling circle amplification (RCA) and multiple displacement amplification (MDA). These techniques are available to identify wide varieties of microorganisms. A LAMP assay has been designed based on the *ITS-2* gene for the identification of *S. scabiei* and has been found to be promising after evaluation of skin scrapings from infected animals with sarcoptic mange that showed 100% sensitivity and 92.3% specificity [8].

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